The immunoproteasome: a novel drug target for autoimmune diseases

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ABSTRACT

The immunoproteasome, a special class of the proteasome, is mainly expressed in cells of haematopoietic origin. Additionally, during inflammation, the immunoproteasome is induced by IFN-γ or TNF-α. In recent years it became apparent that the immunoproteasome has important functions other than processing proteins for MHC class I restricted presentation. The immunoproteasome plays a critical role in T cell expansion, cytokine production, and T helper cell differentiation. Inhibition of the immunoproteasome ameliorated disease symptoms in different animal models for autoimmune diseases. Hence, the unique role for LMP7 in controlling pathogenic immune responses provides a therapeutic rationale for targeting LMP7 in autoimmune disorders. In this review we summarise the effect of immunoproteasome inhibition in animal models for rheumatoid arthritis, inflammatory bowel disease, Hashimoto's thyroiditis, systemic lupus erythematosus, and multiple sclerosis.

Introduction

The proteasome is the central non-lysosomal protein degrading machine in the cytoplasm and nuclei of all eukaryotic cells. The 20S proteasome is a barrel-shaped complex of four rings with seven subunits each. The outer two rings consist of α subunits, the inner two rings of β subunits forming the central proteolytic chamber (1). The β subunits β1, β2, and β5 bear the catalytically active centers of the 20S proteasome. The proteasome is in charge of degrading key proteins in metabolism, cell cycle control, and cell differentiation and has turned out to be an effective target in the treatment of relapsed and/or refractory myeloma and mantle cell lymphoma (2). Additionally, the proteasome is responsible for the generation of most ligands presented on major histocompatibility complex class I molecules (MHC-I) (3, 4). In cells treated with interferon (IFN)-γ and tumour necrosis factor (TNF)-α or in cells of haematopoietic origin the constitutive catalytic subunits β1c, β2c, and β5c are replaced by the inducible subunits low molecular mass polypeptide (LMP)2 (β1i), multicatalytic endopeptidase complex-like (MECL)-1 (β2i), and LMP7 (β5i), respectively, building the so-called immunoproteasome. The past two decades of immunoproteasome research have yielded a host of evidence that the immunoproteasome shapes MHC-I antigen processing and presentation and by this means also the T cell repertoire (5-12). In recent years, it became evident that the immunoproteasome has apart from its role in antigen processing additional functions in expansion and survival of T cells (5, 7, 13, 14), in T helper cell differentiation (15, 16), in brain inflammation (17, 18), in inflammatory cytokine production (10, 15, 18, 19), and in autoimmune diseases (15, 18-23) (Fig. 1).

The proteasome, as a key player in cell cycle progression and immune response, is a promising drug target. However, by blocking both types of proteasomes, the constitutive and the immunoproteasome, all approved drugs exhibit severe toxicity. Therefore, novel proteasome inhibitors preferentially targeting subunits of the immunoproteasome were developed (15, 24-30). Additionally, the recently solved crystal structures of the constitutive proteasome and immunoproteasome at 2.9 Å resolution allows the structure-guided design of inhibitory lead structures selective for immunoproteasomes (1). Immunoproteasome selective inhibitors are targeting the proteasome activity at the site of inflammation and, therefore, the proteasome activity required for housekeep-

ing functions at other sites in the body is not affected. This also explains the lacking side effects of immunoproteasome inhibitors in mice compared to broad-spectrum proteasome inhibitors (15, 18, 19, 22). Hence, the immunoproteasome may qualify as a new treatment option to prevent the progression of autoimmune diseases.

The immunoproteasome in autoimmune diseases

The immunoproteasome has recently been shown to be involved in the pathogenesis of autoimmune diseases and in the modulation of T helper cell differentiation (31). Selective inhibition of the LMP7 (β5i) subunit of the immunoproteasome ameliorated clinical symptoms in mouse models of rheumatoid arthritis (15), multiple sclerosis (18), systemic lupus erythematosus (22), inflammatory bowel disease (19), diabetes (15), and Hashimoto's thyroiditis (23) (Fig. 1). Moreover, LMP7 inhibition or deficiency was shown to suppress the differentiation of the pro-inflammatory T helper subtypes Th1 and Th17 while it enhances the generation of anti-inflammatory regulatory T cells (Tregs) *in vitro* (summarised in (31)). Furthermore, selective inhibition of the immunoproteasome was found to negatively regulate the production of pro-inflammatory cytokines like IL-6, IFN-γ, TNF-α, GM-CSF, and IL-23 (15, 18).

Experimental rheumatoid arthritis

Initially, the therapeutic impact of LMP7 inhibition has been demonstrated in two mouse models of rheumatoid arthritis: collagen antibody–induced arthritis (CAIA) and collagen-induced arthritis (CIA) (15). In the CAIA model, a T cell-independent rheumatoid arthritis model, the mice were challenged with antibodies to type II collagen and endotoxin. Thereby, the LMP7-selective inhibitor ONX 0914 (formerly named PR-957) blocked disease progression in a dose-dependent manner. Inhibition of LMP7 alone was sufficient to block disease progression, as evidenced by the therapeutic response to ONX 0914 administered at 2 mg/kg body weight, that is, less than one-tenth of the maximal tolerated dose (MTD). Anti-inflammatory responses induced by ONX 0914 were rapid and long lasting. A single dose, administered either intravenously or subcutaneously, resulted in marked amelioration of disease. Inflammatory infiltration and subsequent bone erosion were significantly decreased in ONX 0914 treated mice. Compared to anti-TNF-α therapy (etanercept), ONX 0914 treated mice demonstrated a more rapid resolution of clinical symptoms. Similar to the CAIA model, ONX 0914 treatment induced a rapid therapeutic response in the T and B cell-dependent CIA model, in which arthritis is induced with immunisation of mice with type II collagen (15). Immunoproteasome inhibition was associated with a decrease in circulating levels of autoantibodies and collagen oligomeric matrix protein (COMP), a marker for cartilage breakdown. ONX 0914 stabilised disease symptoms and was more effective than etanercept. In contrast to ONX 0914, TNF-α blockage could not reduce autoantibody levels. Similar findings in lymphocyte-dependent and lymphocyte-independent models of rheumatoid arthritis were observed, arguing that ONX 0914 modulates the activity of multiple effector cell types in these disease models. Indeed, ONX 0914 inhibits both T cell and monocyte functions *in vitro*, as seen by altered cytokine release of T cells or monocytes (15, 18). ONX 0914 treatment of human PBMCs derived from patients diagnosed with active rheumatoid arthritis (RA), blocked IL-23 production and TNF-α secretion in immune cells of these patients, suggesting that LMP7 activity in these cells regulates inflammatory cytokine production in cells from both, healthy volunteers and patients with active RA (15).

Inflammatory bowel disease

Inflammatory bowel disease (IBD) refers to a group of chronic inflammatory disorders of the gastrointestinal tract. It has been demonstrated that pharmacological inhibition with the proteasome inhibitor bortezomib reduced colitis development in a rat (32) and a mouse (21) colitis model. Furthermore, blockage of the immunoproteasome subunit LMP7 by the LMP7-selective inhibitor ONX 0914 strongly reduced pathological symptoms of dextran sulfate sodium (DSS)-induced colitis in mice, as determined by weight loss and colon length (19). Production of numerous cytokines in ONX 0914 treated mice was suppressed, resulting in reduced inflammation and tissue destruction. Interestingly, administration of a β5c-selective inhibitor had no effect on colitis severity, indicating that the reduction of pathological symptoms observed in bortezomib treated mice is only dependent on LMP7 (21). Similar to ONX 0914 treated mice, induction of colitis by oral administration of DSS in the drinking water of LMP2-, LMP7-, and MECL-1-deficient mice revealed a partial to complete protection of these mice compared to WT mice (16, 19-21). Both, infiltration of the colon by neutrophils and expansion of inflammatory T helper 1 (Th1) and Th17 T cells was diminished in LMP7-deficient mice thereby preventing excessive tissue damage (16, 21). Interestingly, experiments with bone marrow chimeras revealed that cells of the haematopoietic system are responsible for the protective effect in LMP7-deficient mice (21).

Hashimoto's thyroiditis and Graves' disease

Hashimoto's thyroiditis and Graves' disease are two autoimmune thyroid diseases. Grave's disease is an autoantibody-mediated disease where class II antigen presentation to $CD4^+$ cells is crucial for stimulating B cells and inducing the pathogenic stimulatory anti-thyrotropin receptor (TSHR) autoantibodies. In contrast, Hashimoto's thyroiditis is cell-mediated, where MHC class I and II antigen presentation involving $CD4^+$ and $CD8^+$ T cells is relevant to the generation of antigen-specific cytotoxic T lymphocytes. Recently, the efficacy of the immunoproteasome inhibitor ONX 0914 for the treatment of autoimmune thyroid diseases was investigated using mouse models of cell-mediated Hashimoto's thyroiditis and autoantibody-mediated Graves' hyperthyroidism (23). Autoim-

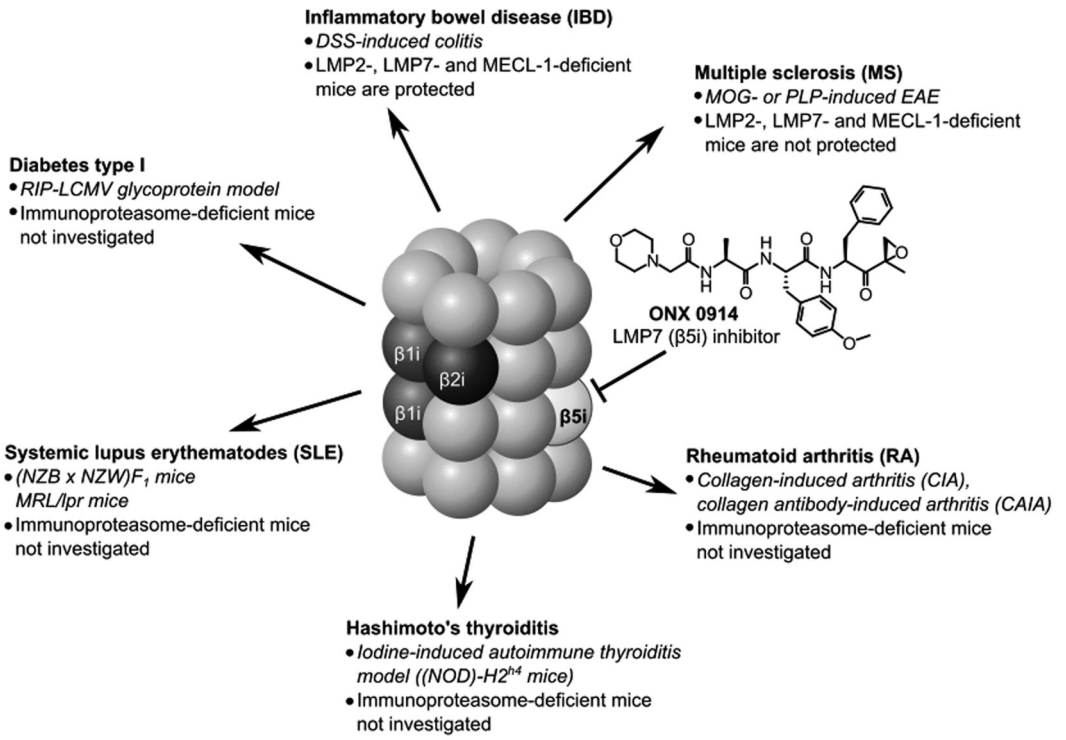

Fig. 1. Immunoproteasome inhibition in autoimmune diseases. Inhibition of the immunoproteasome subunit LMP7 (β5i) in animal models for autoimmune diseases protects against diabetes (15), inflammatory bowel disease (19), multiple sclerosis (18), rheumatoid arthritis (15), Hashimoto's thyroiditis (23), and systemic lupus erythematosus (22). For each disease the investigated animal model and the effect in immunoproteasome-deficient mice is outlined.

mune thyroiditis in mice was induced by iodine administration. Intraperitoneal injection of ONX 0914 dosed between 2 and 25 mg/kg twice a week inhibited the development of thyroiditis in a dose-dependent manner. However, its effect on anti-thyroglobulin (Tg) autoantibody titres was less evident, with significant suppression observed only with 25 mg/kg ONX 0914, a dose slightly below the MTD of 30 mg/kg. A significant decrease in thyroiditis scores but not in anti-Tg antibodies was also observed in mice treated with 25 mg/kg ONX 0914 in a therapeutic setup. Nevertheless, one can assume that at the used concentration of 25 mg/kg also the constitutive proteasome is at least partially blocked. The effect of ONX 0914 was also evaluated in a model for Graves' disease involving immunisation of susceptible BALB/c mice with Ad-TSHR289, a non-replicative recombinant human adenovirus expressing the human thyrotropin receptor (TSHR) A-subunit which efficiently induces hyperthyroidism and thyroid-stimulating antibodies. However, different ONX 0914 application regimens revealed no effect of LMP7 inhibition on Graves' disease (23). The authors hypothesised that ONX 0914 efficacy is higher in Hashimoto's thyroiditis compared to Graves' disease because this compound primarily suppresses MHC class I-mediated, *i.e.* cell-mediated autoimmune responses. Nevertheless, the therapeutic effect of ONX 0914 indicates that other mechanisms independent of antigen presentation, like cytokine release or T helper cell differentiation, play an additional role in this model.

Systemic lupus erythematosus-like disease

Systemic lupus erythematosus (SLE) is a complex autoimmune disease characterised by a dysregulation of multiple arms of the immune system and production of IFN-α and hallmark autoantibodies. Proteasome inhibition blocks anti-apoptotic nuclear factor kappa B (NF-κB) activation leading to an accumulation of misfolded proteins within the endoplasmic reticulum (33). Due to their extremely high rate of antibody synthesis, plasma cells are particularly sensitive to proteasome inhibition. Indeed, treatment of two mouse strains with lupus-like disease, NZB/W F1 and MRL/lpr mice, with the broad spectrum proteasome inhibitor bortezomib depleted plasma cells producing antibodies to double-stranded DNA, eliminated autoantibody production, ameliorated glomerulonephritis, and prolonged survival (34). To investigate whether bortezomib induces clinically relevant plasma cell depletion in patients with active, refractory SLE, twelve patients were treated with bortezomib in a clinical trial (35). Strikingly, upon proteasome inhibition, disease activity was reduced and so were serum antibody levels. Bortezomib reduced the numbers of plasma cells in the peripheral blood and bone marrow. These results indicate that bortezomib is clinically effective in refractory SLE, but this needs to be confirmed in controlled trials. Nevertheless, to overcome resistance to bortezomib and to use proteasome inhibitors with improved pharmacological and toxicological profiles, the investigation of immunoproteasome inhibitors in animal models of SLE is warranted. Indeed, treatment of lupus-prone mice with the immunoproteasome-selective inhibitor ONX 0914 prevented disease progression and treatment of mice with established disease attenuated nephritis (22). Levels of total IgG and anti-dsDNA IgG antibody in the serum declined during treatment and cor-

related with a decrease in plasma cell numbers in spleen and bone marrow even after short-term treatment. Furthermore, Ichikawa et al. demonstrated that LMP7 inhibition blocked the production of IFN-α by TLR-activated plasmacytoid dendritic cells (PDC) *in vitro* and *in vivo*, an effect mediated by inhibition of both, PDC survival and PDC function (22). Taken together, inhibition of the immunoproteasome prevents lupus disease progression by targeting two critical pathways in SLE, namely, type I IFN activation and autoantibody production by plasma cells.

Experimental autoimmune encephalomyelitis

Experimental autoimmune encephalomyelitis (EAE) is an animal model for multiple sclerosis (MS) which shares clinical and pathological features with this complex chronic immune-mediated disease of the central nervous system in humans. In this model, sensitisation with central nervous system (CNS) antigens breaks immunological tolerance of autoreactive T cells, enabling them to cross the blood-brain barrier and induce brain inflammation. Recently, the effect of the immunoproteasome in this autoimmune disease model was thoroughly investigated (18). Immunoproteasome inhibition with the LMP7-selective inhibitor ONX 0914 attenuated disease progression after active and passive induction of EAE, both in myelin oligodendrocyte glycoprotein $(MOG)_{35-55}$- and proteolipid protein $(PLP)_{139-151}$-induced EAE (18). Isolation of lymphocytes from the brain or spinal cord revealed a strong reduction of cytokine-producing $CD4^+$ cells in ONX 0914 treated mice. An analysis of draining lymph nodes after induction of EAE revealed that the differentiation to Th1 or Th17 cells was strongly impaired in ONX 0914 treated mice. Isolation of lymphocytes from the brain or the spinal cord at the peak of the disease demonstrated barely any detection of IL-17, IFN-γ, TNF-α, or GM-CSF producing $CD4^+$ cells in ONX 0914 treated as compared to vehicle treated mice. T cell-derived GM-CSF sustains neuroinflammation via myeloid cells that infiltrate the CNS, and IL-23 is necessary for the induction of EAE in mice due to its role in the maintenance of Th17 cells (36, 37). ONX 0914 reduced GM-CSF production of activated mouse T cells under GM-CSF polarising and non-polarising conditions and of human PBMCs stimulated via the T cell receptor (18). Using bone marrow chimeras, the authors could demonstrate that the effect of ONX 0914 treatment relies on the selective inhibition of LMP7 in cells derived from the haematopoietic system. Interestingly, EAE induction in immunoproteasome-deficient mice is similar to wild type mice (18, 38, 39). The question why an LMP7-selective inhibitor is so effective in attenuating symptoms of EAE while LMP7-deficient mice are susceptible to the disease was addressed by inducing EAE in LMP7-deficient mice which were treated with PR-825, a selective inhibitor of β5c (18). While the β5c-selective inhibitor PR-825 failed to prevent disease induction in wild type mice, LMP7-deficient mice treated with the same inhibitor were almost completely protected from MOG_{35-55}-induced EAE. Hence, the ablation of the chymotrypsin-like activity of the proteasome, exerted by β5c and/or β5i, is responsible for the observed beneficial effects of the LMP7-selective inhibitor ONX 0914 in EAE. It seems that the cells responsible for the induction of EAE express high levels of immunoproteasomes and inhibition of LMP7 (β5i) abrogates their proteasomal chymotrypsin-like activity to a degree which interferes with their pathogenic activity. Consecutive episodes of remission and relapse are hallmarks of MS in the majority of patients. Strikingly, ONX 0914 treatment also prevented disease exacerbation in a relapsing-remitting EAE model. LMP7 inhibition ameliorated a relapse when treatment started in the recovery phase after the first wave of symptoms (18). Hence, LMP7 inhibition may qualify as a new treatment option to prevent the progression of autoimmune diseases like MS.

Conclusions

The LMP7-selective inhibitor ONX 0914 is the first proteasome inhibitor described that is selective for the chymotrypsin-like subunit of the immunoproteasome and represents a powerful tool for understanding the role of LMP7 in immune responses. Indeed, the therapeutic impact of ONX 0914 in mouse models of rheumatoid arthritis, inflammatory bowel disease, Hashimoto's thyroiditis**, s**ystemic lupus erythematosus, diabetes, colitis, and multiple sclerosis is intriguing (Fig. 1). Why is LMP7 inhibition so efficient in reducing disease symptoms in these pre-clinical animal models? Two major pathways involved in disease development are affected by LMP7 inhibition, namely cytokine secretion and T helper cell differentiation. LMP7 inhibition of TCR activated T cells and endotoxin stimulated human PBMCs or mouse splenocytes strongly reduced the secretion of numerous pro-inflammatory cytokines (10, 15, 18, 19, 22). Additionally, LMP7 inhibition prevents the differentiation of naïve T helper cells to polarised Th1 or Th17 cells *in vitro* (15, 16). Furthermore, generation of TCR stimulated T cells under GM-CSF polarising conditions is strongly inhibited by ONX 0914 (18). GM-CSF is a potent pro-inflammatory cytokine that is responsible for the recruitment, maturation, and activation of innate immune cells. In this regard, GM-CSF production by T cells has been associated with several autoimmune diseases, such as multiple sclerosis and rheumatoid arthritis. The relevance of the IL-23/IL-17 axis in the pathogenesis of autoimmunity is well established. By blocking cytokine secretion, like IL-23 or IL-6 production (15, 18, 19), LMP7 inhibition could influence Th17 differentiation. How can LMP7 inhibition affect cytokine secretion and T helper cell polarisation? Recently, we proposed that the immunoproteasome might selectively processes a factor that is required for regulating cytokine production and T helper cell differentiation (31). Nevertheless, such a factor has not been identified so far. The recent observation that similar to LMP7 inhibition in wild type mice, β5c inhibition in LMP7-deficient mice ameliorates EAE argues against a factor selectively processed by the immunoproteasome (18). Additionally, cytokine secretion

can be inhibited with a β5c selective inhibitor in LMP7-deficient cells (19). Hence, it rather seems that the activation of immune cells is impaired in cells containing proteasomes with blocked chymotrypsin-like activity. Bortezomib inhibits NF-κB activation by blocking the degradation of the NF-κB inhibitor (IκB), leading to reduced expression of pro-inflammatory factors, such as cytokines. This implies that the observed effects in multiple models of autoimmune diseases might be due to an inhibition of the NF-κB pathway. Nevertheless, LMP7-blockage does not inhibit NF-κB activity in a reporter cell line at selective concentrations, suggesting that LMP7 regulates cytokine production via NF-κB–independent pathways (15). Thus, the mechanism how the blockage of the chymotrypsin-like activity of the proteasome alters cytokine secretion and T helper cell differentiation remains to be elucidated. Interestingly, immunoproteasome-deficient mice are protected from DSS-induced colitis. Hence, in this autoimmune model a different, immunoproteasome-specific mechanism must enable protection from the disease.

The promising results obtained in preclinical autoimmune models render the immunoproteasome an interesting drug target for the treatment of autoimmune diseases in humans and a transfer of immunoproteasome-selective inhibitors into the first clinical trials is highly warranted.

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